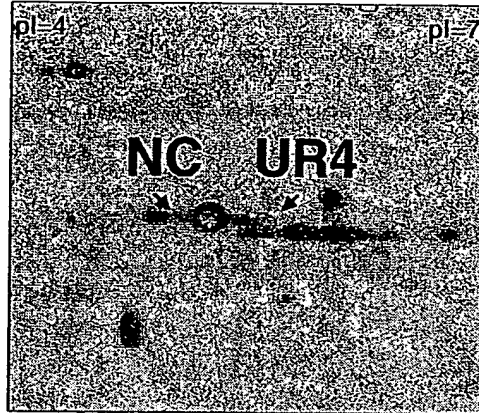


(A)

CEM



(B)

CEM/VLB

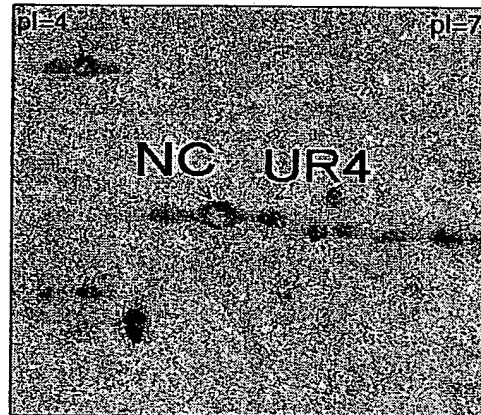


Figure 1

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(A) Isoform 2 Results

ProFound - Search Result Summary						Version 4.10.8 © 1997-2000 ProteoMetrics				
Protein Candidates for search 20010603200436-0394-208172123151 (73182 sequences searched)										
Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (T)				%	pi	kDa	®
1	1.0e+000	2.36	[5729877]reflNP_006588.1 heat shock 70kD protein 3; heat shock 70kD protein 3 (HSP73); heat shock cognate protein 71 kDa; heat shock 70kD protein 10 (HSC71) [Homo sapiens]				25	5.4	71.11	®

(B) Search Parameters

Details for rank 1 candidate in search 20010603200436-0394-208172123151	
[5729877]reflNP_006588.1 heat shock 70kD protein 3; heat shock 70kD protein 3 (HSP73); heat shock cognate protein 71 kDa; heat shock 70kD protein 10 (HSC71) [Homo sapiens]	
[2639862]refXP_006036.2 heat shock 70kD protein 3 [Homo sapiens]	
[123648]refPII_4 HSC70 HUMAN HEAT SHOCK COGNATE 71 KDA PROTEIN	
[87629]refA27077 dnaK-type molecular chaperone human	
[32467]embCAA68445.1 [G00371] 71 kD heat shock cognate protein [Homo sapiens]	
[13273304]gdbAAK17893.1 AF352832.1 (AF352832) constitutive heat shock protein 70 [Homo sapiens]	
Sample ID: NO CHANGE (Pass 0)	
Measured peptides: 20	
Matched peptides: 12	
Min. sequence coverage: 25%	

(C)

12 Peptides Sequenced*	Measured Avg/		Computed Mass	Error (ppm)	Residues Missed			Peptide sequence
	Mass (M)	Mono			Start	To	Cut	
-1	1250.623	M	1250.611	10	237	246	0	MVNHFIAEFK (1)+O@M;
-2	1252.592	M	1252.608	-13	302	311	0	FEELNADLFR
-3	1406.696	M	1406.712	-12	237	247	1	MVNHFIAEFKR (1)+O@M;
-4	1479.743	M	1479.746	-2	300	311	1	ARFEELNADLFR
-5	1486.756	M	1486.693	42	37	49	0	TTPSYVAFTDTER
-6	1690.705	M	1690.718	-7	221	236	0	STAGDTHLGGEDFDNR
-7	1786.968	M	1786.982	-8	172	188	1	IINEPTAAAIAYGLDKK
-8	1820.874	M	1820.883	-5	57	72	1	NQVAMNPNTVFDAGR (1)+O@M;
-9	1837.001	M	1837.005	-2	326	342	1	LDKSQIHDIVLVGGSTR
-10	1951.065	M	1951.052	7	452	469	1	DNNLLGKFELTGI PPAPR
-11	1980.996	M	1980.990	3	138	155	0	TVTNAVVTVPAYFNDSQR
-12	2773.258	M	2773.318	-21	424	447	0	QTQTFTTYSDNQPGVLIQVYEGER

*25% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed.

Figure 2

1 MSKGPVAVGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVA
61 MNPTNTVFDKRLIGRRFDDAVVQSDMKHWPFMVVNDAGRPKVQVEYKGETKSFYPEEVS
121 SMVLTKMKEIAEAYLGKTVTNAVVTVPAYFNDSQRQATKDAGTIAGLNVLRIINEPTAAA
181 IAYGLDKKVGAEARNVLIFFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRMVNH
241 FIAEFKRKHKKDISENKRAVRRRLRTACERAKRTLSSSTQASIEIDSLYEGIDFYTSITRA
301 RFEELNADLFRGTLDPVEKALRDAKLDKSIHQDIVLVGGSTRIPKIQKLLQDFFNKELN
361 KSINPDEAVAYGAAVQAAILSGDKSENVQDLLLDVTPLSLGIETAGGVMTVLIKRNTTI
421 PTKQTQTFTTYSNQPGLVLIQVYEGERAMTKDNNLLGKFELTGIPPAPRGVVPQIEVTFDI
481 DANGILNVSVDKSTGKENKITITNDKGRLSKEDIERMVQEAKEYKAEDEKQRDKVSSKN
541 SLESYAFNMKATVEDEKLQGGKINDEKQKILDKCNIEINWLDKNQTAEKEEFEHQQKELE
601 KVCNPIITKLYQSAGGMPGGMPGGFPGGGAPPSGGASSGPTIEEVD

BOLD = Peptides covered by MS MALDI analysis

Figure 3

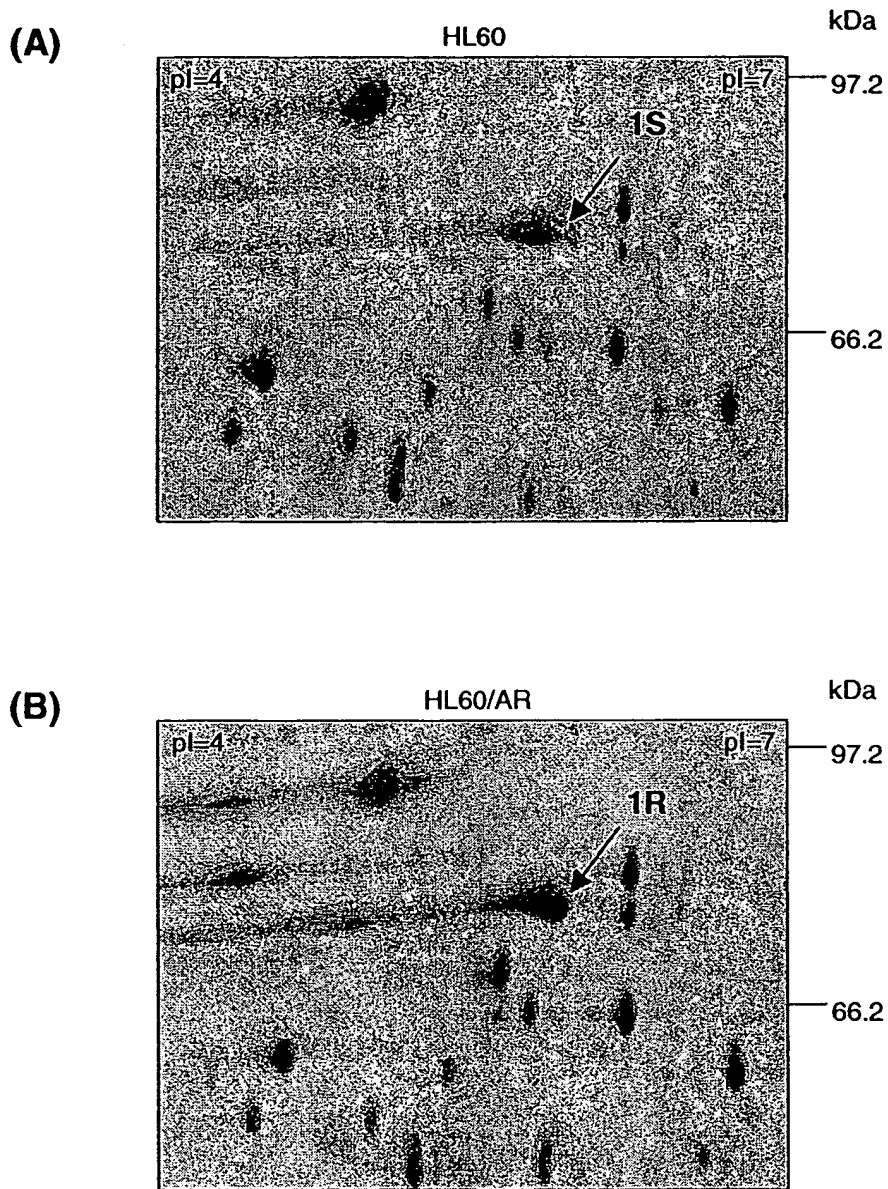


Figure 4

(A)

ProFound Search Result Summary						Version 4.10.3 The Rockefeller University Edition
Protein Candidates for Search BD96B559-055C-2A0FB235 [98967 sequences searched]						
Rank	Probability	Est'd Z	Protein Information and Sequence Analysis Tools (T)			% pI kDa
1	1.0e-000	2.43	1	5729877	trcBNP_006588.1 (NM_006597)	heat shock 70kD protein 8 (heat shock 70kD protein 8 (HSP73))
2			26	54	71.11	100
[Homo sapiens]						

(B)

Search Parameters

Input Summary	
Date & Time	Mon Dec 17 01:01:33 2001 UTC (Search Time 2.80 sec)
Sample ID	
Database	NCBItr (2001/12/11)
Taxonomy Category	Homo sapiens (human)
Protein Mass Range	60 - 90 kDa
Protein pI Range	4.0 - 5.5
Search for	Single protein only
Digest Chemistry	Trypsin
Max Missed Cut	2
Modifications	+C2H3ON@C(Complete), +O@M(Partial) +C22H37N4O4S@K(Partial)
Charge State	MH
Peptide Masses (Da Average)	
Tolerance (AVG)	100.00 ppm
Peptide Masses (Da Monoisotopic)	1199.667, 1253.610, 1401.805, 1407.785, 1463.128, 1480.751, 1487.698, 1691.729, 1830.802, 1838.043, 1966.104, 1982.001, 1994.000, 2231.141, 2275.180, 2278.081, 2757.251, 2774.246, 2998.417, 3548.224
Tolerance (MON)	100.00 ppm
Number of Peptides	20

(C)

12 Peptides Sequenced*

Measured peptides	20
Matched peptides	12
Min. sequence coverage	26%

Boxed peptides are biotinylated

-1	1198.659	M	1198.666	-6	160	171	0	DRGTLAGLNVLR
-2	1252.602	M	1252.608	-5	302	311	0	FEELNADLFR
-3	1406.717	M	1406.712	46	237	247	3	MYNHTLAETKR
-4	1479.744	M	1479.746	-2	300	311	1	ARPEELNADLFR
-5	1486.690	M	1486.693	-2	37	49	0	TFPSYVAFTDTR
-6	1690.721	M	1690.716	2	221	236	0	STAGDTHLGGEDTNR
-7	1837.035	M	1837.005	16	326	342	3	LDKSOIHDIWVGGSFR
-8	1965.096	M	1965.083	7	312	325	2	GTLDPEKALRDAK
-9	1980.994	M	1980.990	-2	138	155	0	IVTNAVVTVPAYFNDSQK
-10	1980.994	M	1980.956	19	518	533	3	MVOEAEKQKADEKQR
-11	2274.772	M	2274.736	16	57	72	2	NOVAMIPENTVFAKQR
-12	2773.238	M	2773.318	-29	424	447	0	OTOTETTYSDNQPGLIQVYEGER
-13	2997.409	M	2997.452	-24	540	557	3	NSLESYAFIMKATVEDEK

*26% of the amino acids of the HSC70 protein were represented in the amino acids of the mass peptides analyzed. Note that 2 and 4 represent the same peptide

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Figure 5

1 MSKGPVAVGIDLGTTYSCVGVFQHGKVEIANDQGNRTTPSYVAFTDTERLIGDAAK**NQVA**
61 **MNPTNTVFDKRL**LIGRRFDDAVVQSDMKHWPFMVNDAGRPKVQVEYKGETKSFYPEEVS
121 SMVLTKMKEIAEAYLGKTVTNAVVTVPAYFNDSQRQATKDAGTIAGLNVLRIINEPTAAA
181 IAYGLDKKVGAEARNVLIFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRMVNH
241 FIAEFKRKHKKDISENKRAVRRRLRTACERAKRTLSSSTQASIEIDSLYEGIDFYTSITRA
301 RFEELNADLFR**GTLDPVEKALRDAKL**DKSQIHDIVLVGGSTRIPKIQKLLQDFFNGKELN
361 KSINPDEAVAYGAAVQAAILSGDKSENVQDLLLLDVTPLSLGIETAGGVMTVLIKRNTTI
421 PTKQTQTFTTYSDNQPGVLIQVYEGERAMTKDNNLLGKFELTGIPPAPRGVPPQIEVTFDI
481 DANGILNNSAVDKSTGKENKITITNDKGRLSKEDIERMVQEAKEYKAEDEKQRDKVSSK**M**
541 **SLESYAFNMKATVEDEK**LQGKINDEDKQKILDKCNEIINWLDKNQTAEKEEFEHQKKELE
601 KVCNPIITKLYQSAGGMPGGMPGGFPGGGAPPSGGASSGPTIEEVD

BOLD = Nonbiotinylated peptides specific for HSC70
Underlined & Italics = Biotinylated peptides

Figure 6

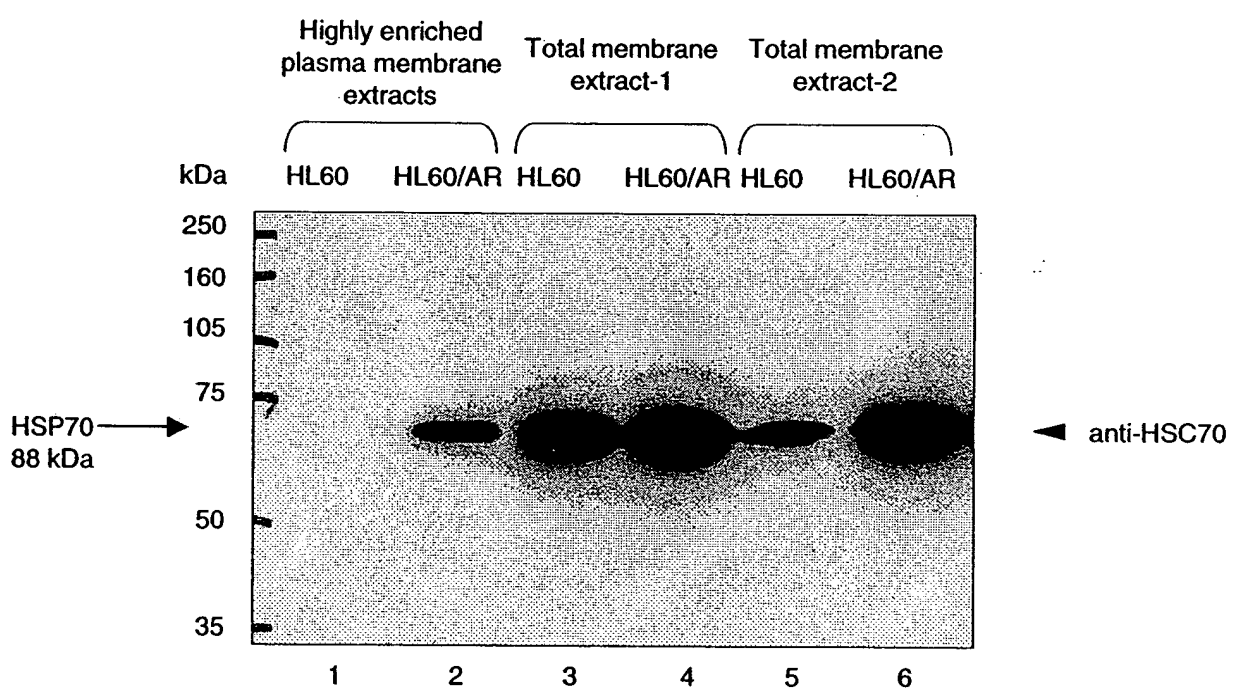
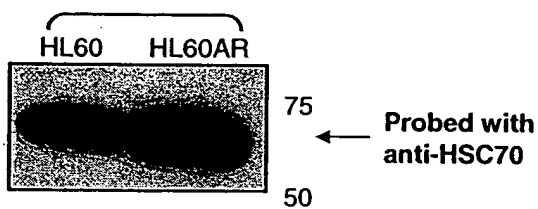


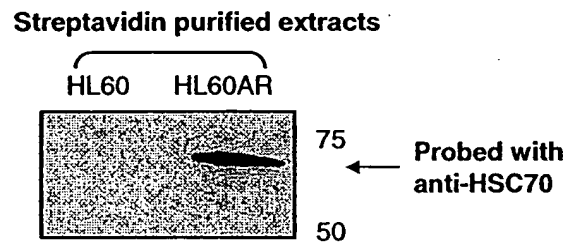
Figure 7

Biotinylated total cell extracts



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Figure 8A



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Figure 8B

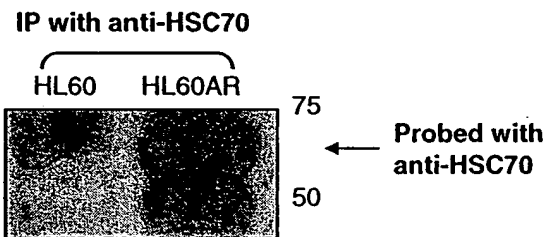
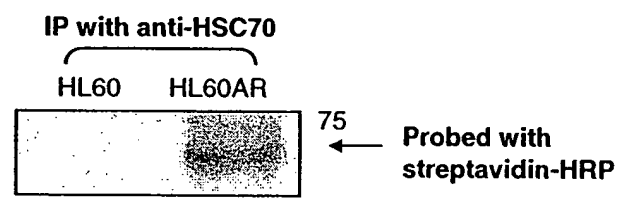


Figure 8C

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Figure 8D

Biotinylated total cell extracts

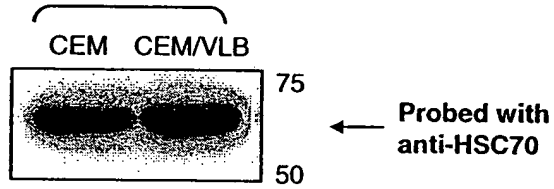


Figure 9A

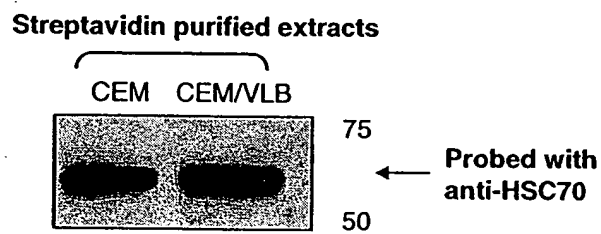


Figure 9B

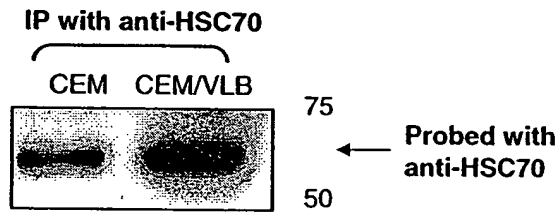


Figure 9C

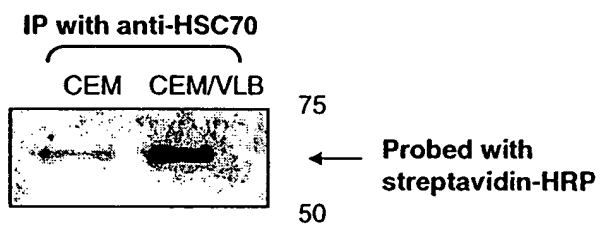


Figure 9D

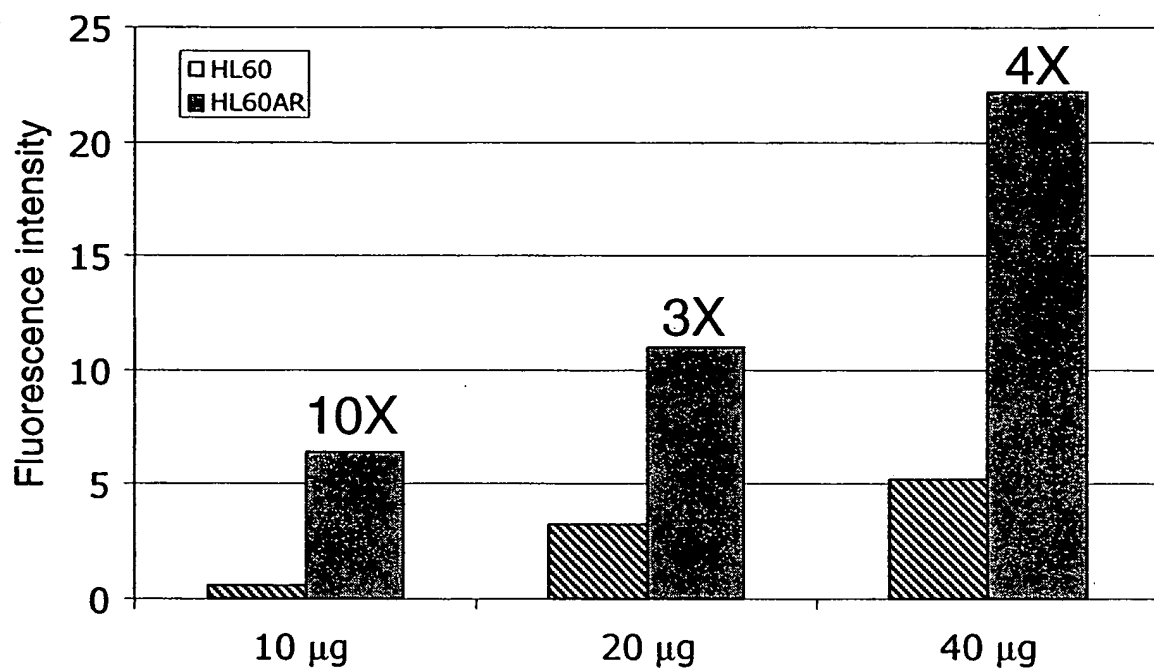


Figure 10A

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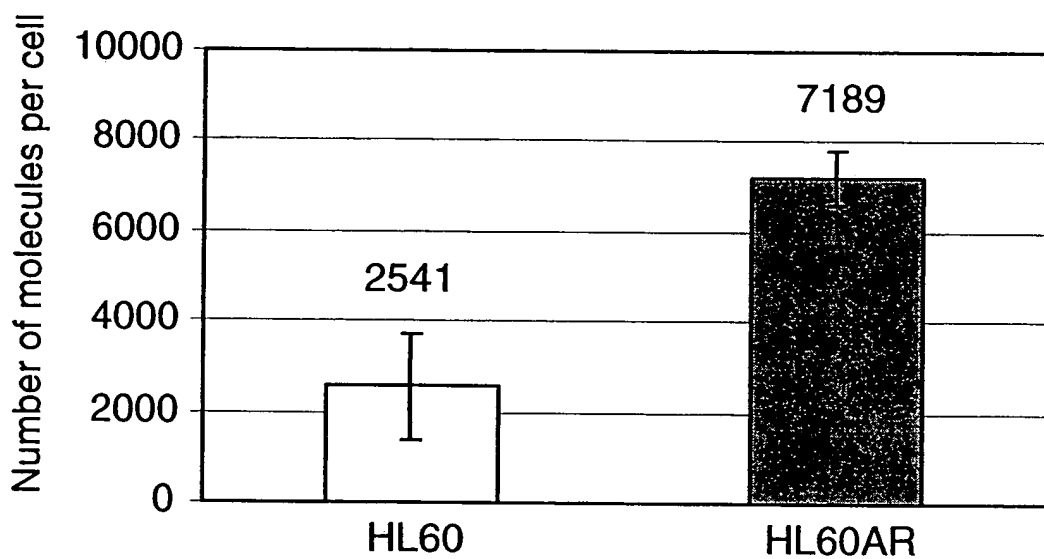


Figure 10B

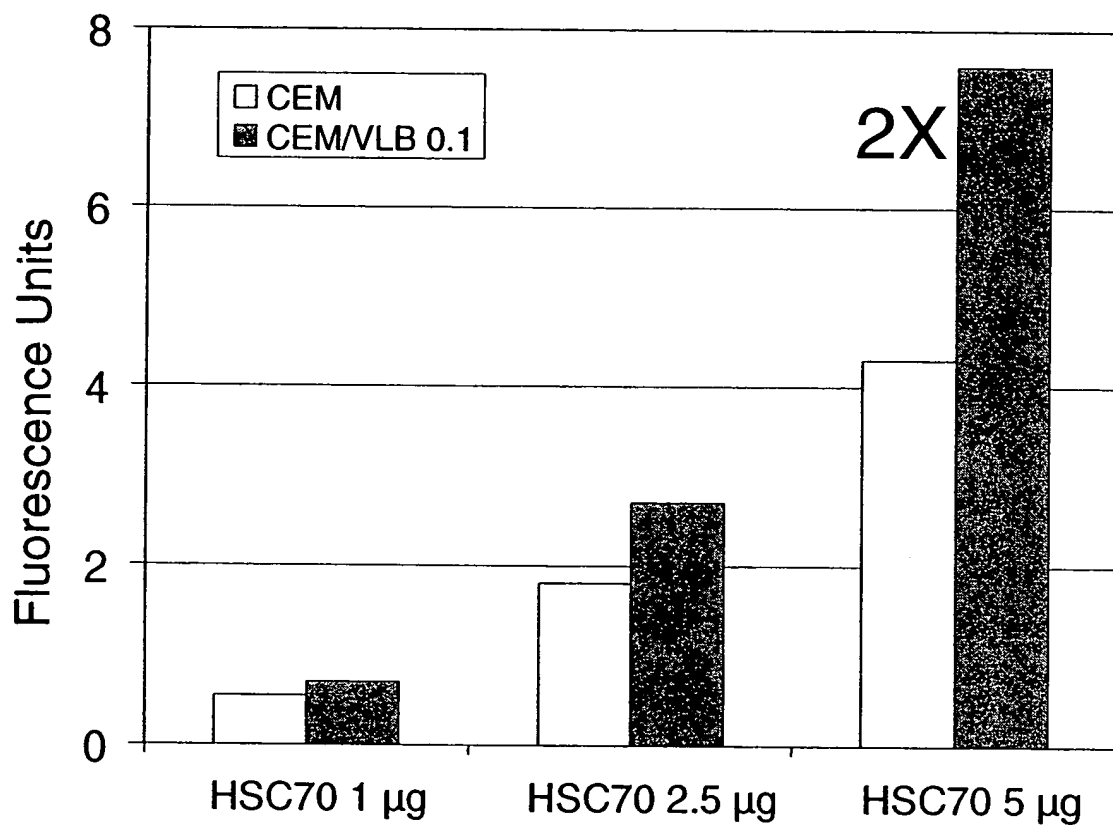


Figure 11A

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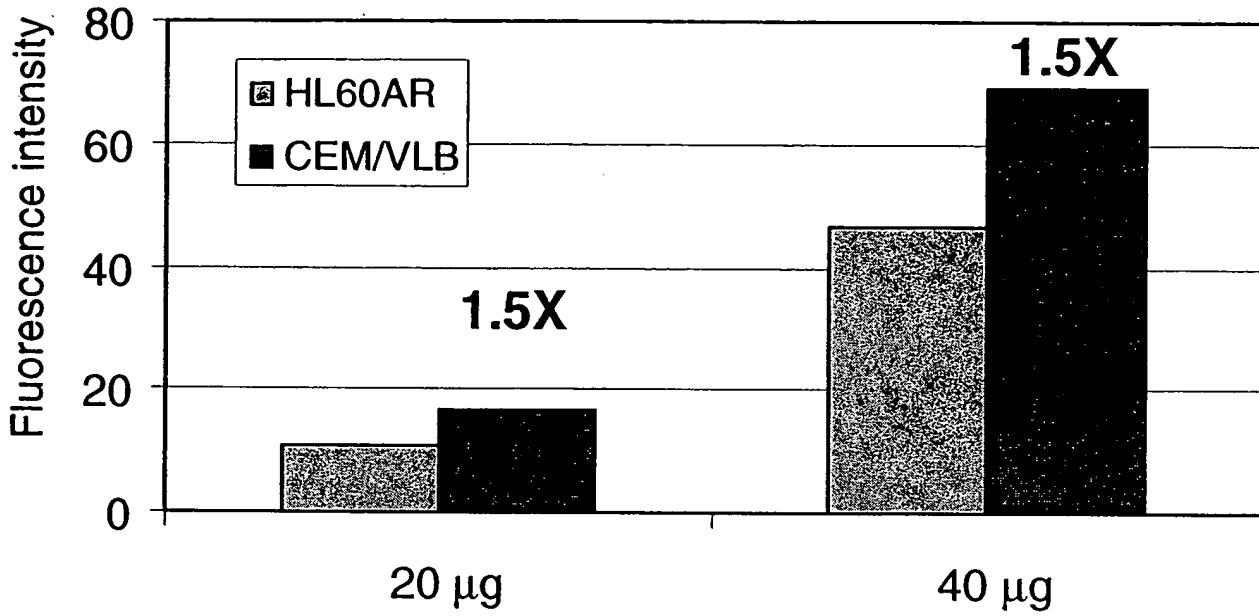


Figure 11B BEST AVAILABLE COPY

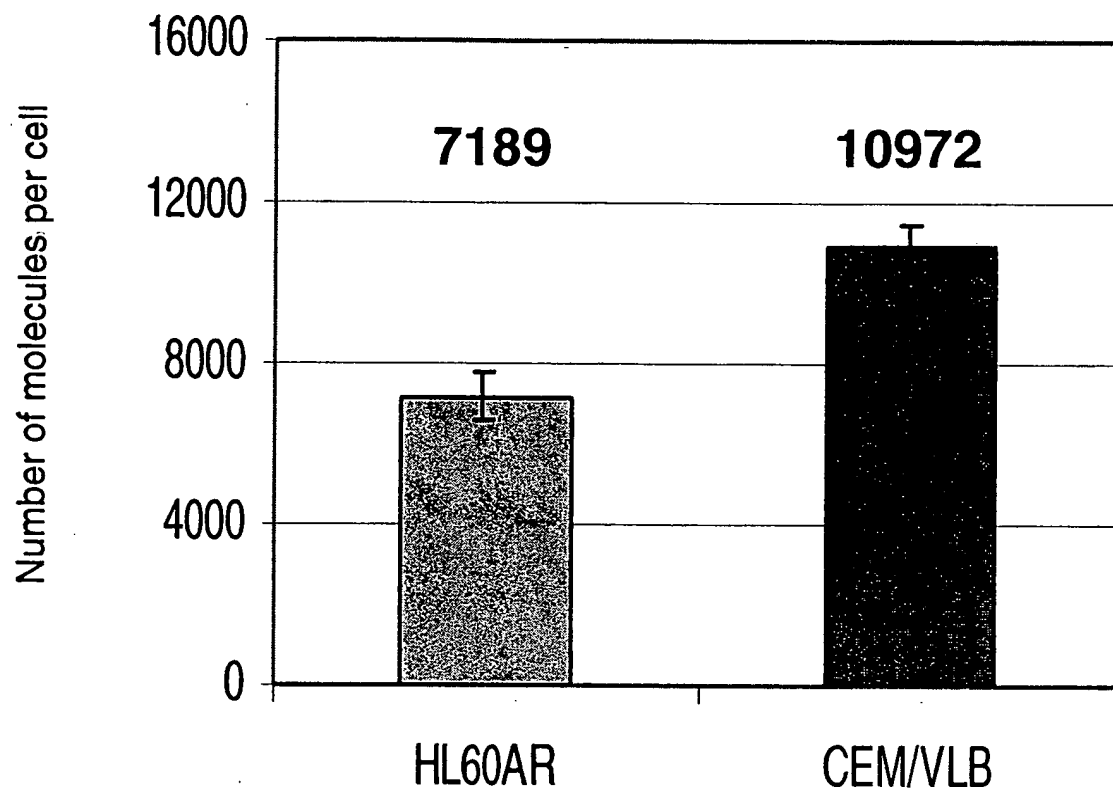


Figure 11C

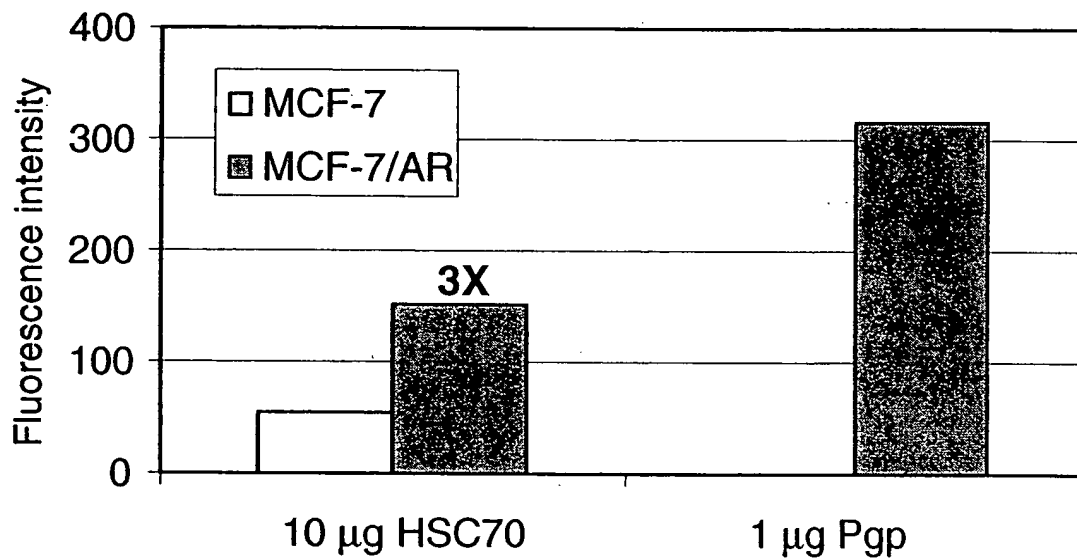


Figure 12A

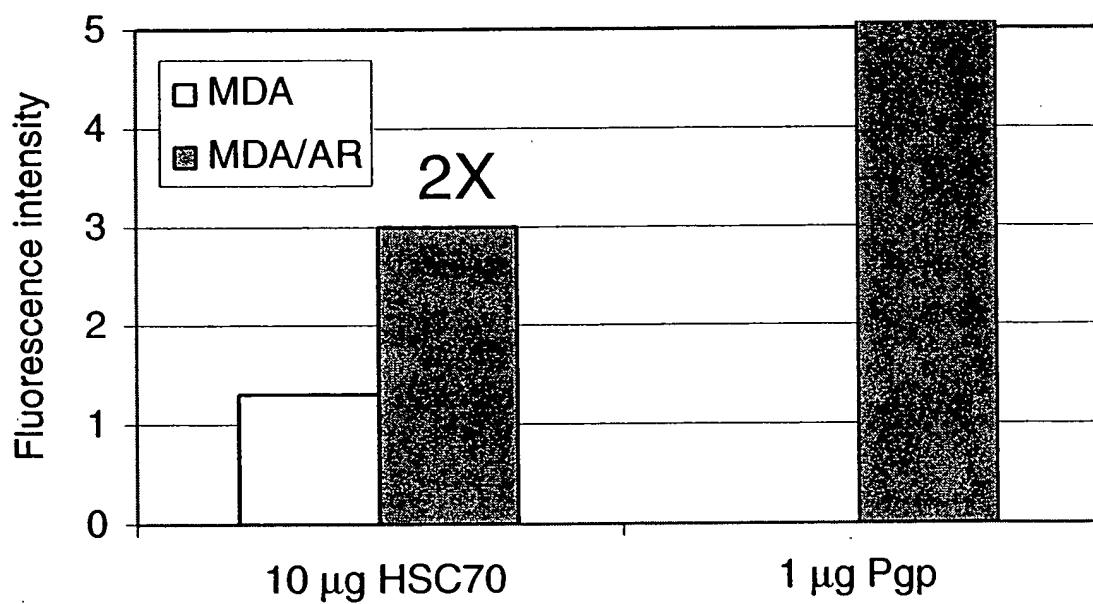


Figure 12B BEST AVAILABLE COPY

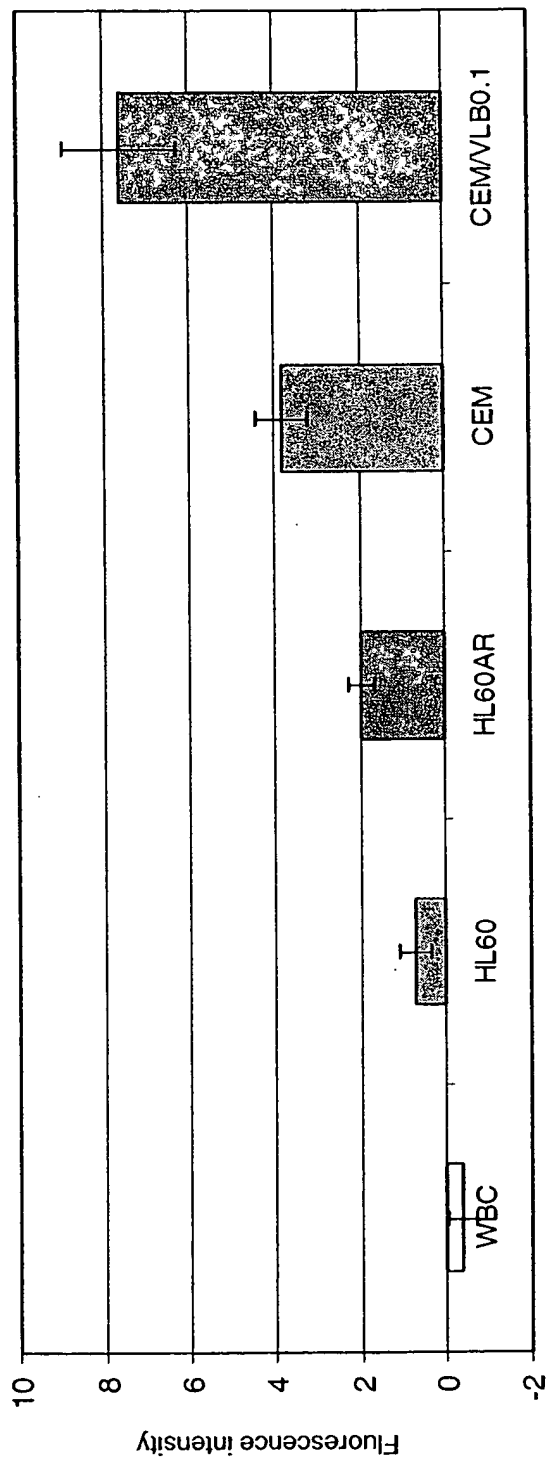


Figure 13A

FIGURE 14

A

POLYPEPTIDE SEQUENCE OF HUMAN HSC70

(GENBANK ACCESSION NO. AAK17898 (SEQ ID NO. 1))

1 MSKGPVAVGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL IGDAAKNQVA
61 MNPTNTVFDA KRLIGRRFDD AVVQSDMKHW PFMVNDAGR PKVQVEYKGE TKSFYPEEVS
121 SMVLTKMKEI AEAYLGKTVT NAVVTVPAYF NDSQRQATKD AGTIAGLNVL RIINEPTAAA
181 IAYGLDKKVG AERNVLIFDL GGGTFDVSIL TIEDGIFEVK STAGDTHLGG EDFDNRMVNH
241 FIAEFKRKHK KDISENKRAV RRLRTACERA KRTLSSSTQA SIEIDSLYEG IDFYTSITRA
301 RFEELNADLF RGTLDPVEKA LRDAKLDKSQ IHDIVLVGGS TRIPKIQKLL QDFFNKGKELN
361 KSINPDEAVA YGAAVQAAIL SGDKSENVQD LLLLDVTPLS LGIETAGGVM TVLIKRNTTI
421 PTKQTQFTTT YSDNQPGVLI QVYEGERAMT KDNLLGKFE LTGIPPAPRG VPQIEVTFDI
481 DANGILNVSA VDKSTGKENK ITITNDKGRL SKEDIERMVQ EAEKYKAEDE KQRDKVSSKN
541 SLESYAFNMK ATVEDEKLQG KINDEDKQKI LDKCNEIINW LDKNQTAEKE EFEHQQKELE
601 KVCNPIITKL YQSAGGMPGG MPGGFPGGGA PPSGGASSGP TIEEVD

B

DNA SEQUENCE OF HUMAN HUMAN HSC70

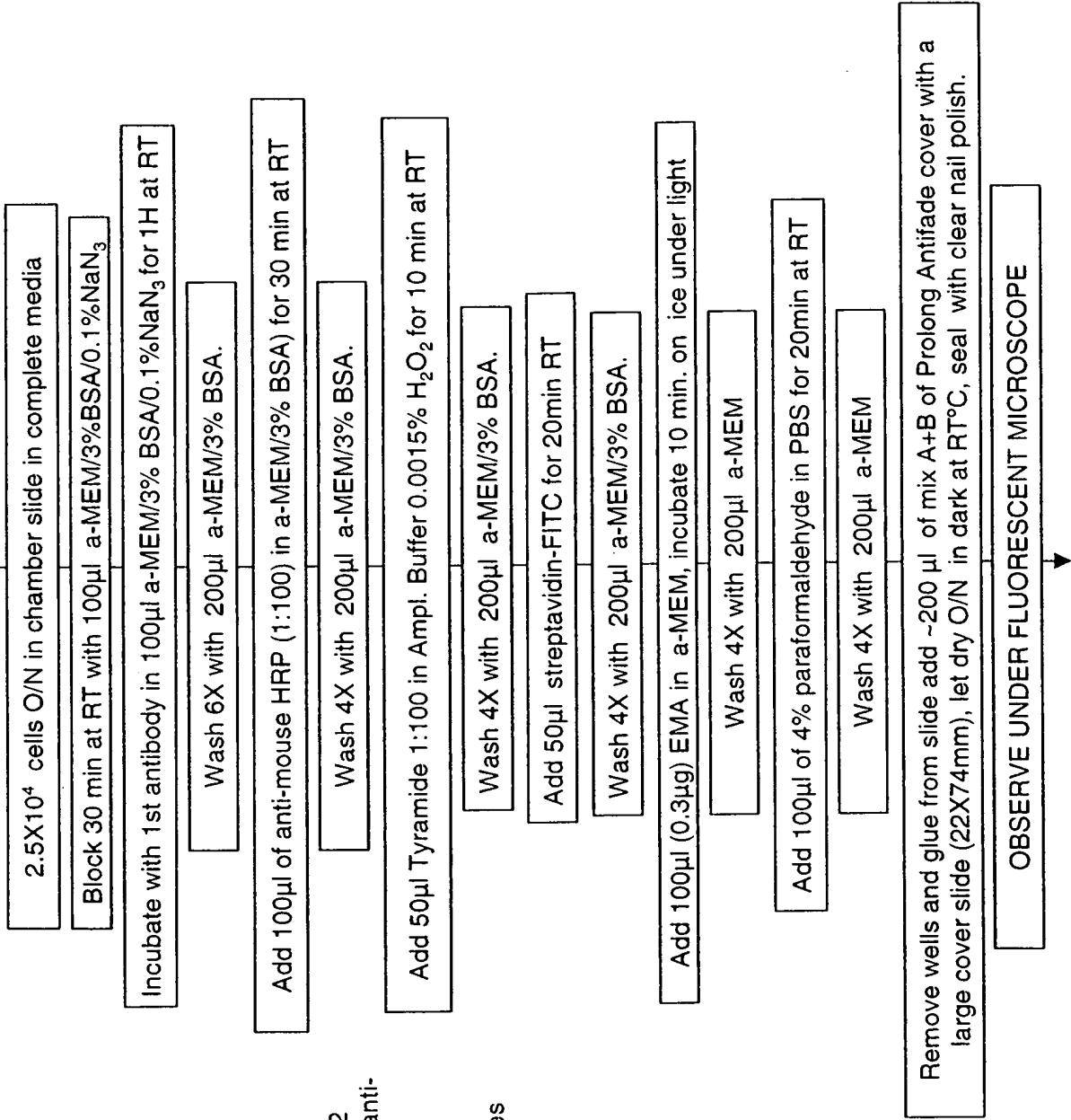
(GENBANK ACCESSION NO. AF352832 (SEQ ID NO. 2))

1 ATGTCCAAGG GACCTGCAGT TGGTATTGAT CTTGGCACCA CCTACTCTTG TGTGGGTGTT
61 TTCCAGCACG GAAAAGTCGA GATAATTGCC AATGATCAGG GAAACCGAAC CACTCCAAGC
121 TATGTCGCCT TTACGGACAC TGAACGGTTG ATCGGTGATG CCGCAAAGAA TCAAGTTGCA
181 ATGAACCCCA CCAACACAGT TTTTGATGCC AAACGTCTGA TTGGACGCAG ATTTGATGAT
241 GCTGTTGTCC AGTCTGATAT GAAACATTGG CCCTTTATGG TGGTGAATGA TGCTGGCAGG
301 CCCAAGGTCC AAGTAGAATA CAAGGGAGAG ACCAAAAGCT TCTATCCAGA GGAGGTGTCT
361 TCTATGGTTC TGACAAAGAT GAAGGAAATT GCAGAAGCCT ACCTTGGGAA GACTGTTACC

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421 AATGCTGTGG TCACAGTGCC AGCTTACTTT AATGACTCTC AGCGTCAGGC TACCAAAGAT
481 GCTGGAAC TA TTGCTGGTCT CAATGTACTT AGAATTATTA ATGAGCCAAC TGCTGCTGCT
541 ATTGCTTACG GCTTAGACAA AAAGGTTGGA GCAGAAAGAA ACGTGCTCAT CTTTGACCTG
601 GGAGGTGGCA CTTTTGATGT GTCAATCCTC ACTATTGAGG ATGGAATCTT TGAGGTCAAG
661 TCTACAGCTG GAGACACCCA CTTGGGTGGA GAAGATTTTG ACAACCGAAT GGTC AACCAT
721 TTTATTGCTG AGTTTAAGCG CAAGCATAAG AAGGACATCA GTGAGAACAA GAGAGCTGTA
781 AGACGCCTCC GTACTGCTTG TGAACGTGCT AAGCGTACCC TCTCTTCCAG CACCCAGGCC
841 AGTATTGAGA TCGATTCTCT CTATGAAGGA ATCGACTTCT ATACCTCCAT TACCCGTGCC
901 CGATTTGAAG AACTGAATGC TGACCTGTTT CGTGGCACC TGGACCCAGT AGAGAAAGCC
961 CTTGAGATG CCAAAC TAGA CAAGTCACAG ATTCATGATA TTGTCCTGGT TGGTGGTTCT
1021 ACTCGTATCC CCAAGATTCA GAAGCTTCTC CAAGACTTCT TCAATGGAAA AGAACTGAAT
1081 AAGAGCATCA ACCCTGATGA AGCTGTTGCT TATGGTG CAG CTGTCCAGGC AGCCATCTTG
1141 TCTGGAGACA AGTCTGAGAA TGTTC AAGAT TTGCTGCTCT TGGATGTCAC TCCTCTTCC
1201 CTTGGTATTG AAAC TGCTGG TGGAGTCATG ACTGTCCTCA TCAAGCGTAA TACCACCATT
1261 CCTACCAAGC AGACACAGAC CTTCACTACC TATTCTGACA ACCAGCCTGG TGTGCTTATT
1321 CAGGTTTATG AAGGCGAGCG TGCCATGACA AAGGATAACA ACCTGCTTGG CAAGTTTGAA
1381 CTCACAGGCA TACCTCCTGC ACCCCGAGGT GTTCCTCAGA TTGAAGTCAC TTTTGACATT
1441 GATGCCAATG GTATACTCAA TGTCTCTGCT GTGGACAAGA GTACGGGAAA AGAGAACAAG
1501 ATTACTATCA CTAATGACAA GGGCCGTTT AGCAAGGAAG ACATTGAACG TATGGTCCAG
1561 GAAGCTGAGA AGTACAAAGC TGAAGATGAG AAGCAGAGGG ACAAGGTGTC ATCCAAGAAT
1621 TCACTTGAGT CCTATGCCTT CAACATGAAA GCAACTGTTG AAGATGAGAA ACTTCAAGGC
1681 AAGATTAACG ATGAGGACAA ACAGAAGATT CTGGACAAGT GTAATGAAAT TATCAACTGG
1741 CTTGATAAGA ATCAGACTGC TGAGAAGGAA GAATTTGAAC ATCAACAGAA AGAGCTGGAG
1801 AAAGTTTGCA ACCCCATCAT CACCAAGCTG TACCAGAGTG CAGGAGGCAT GCCAGGAGGA
1861 ATGCCTGGGG GATTTCCCTGG TGGTGGAGCT CCTCCCTCTG GTGGTGCTTC CTCAGGGCCC
1921 ACCATTGAAG AGGTTGATTA A

Figure 15A : Procedure for immunofluorescence (non-permeabilized cells)



Amplification kit used: TSA kit #2 with HRP-goat anti-mouse IgG and Alexa fluor 488 tyramide from molecular probes T-20192

Figure 15B: Procedure for immunofluorescence (permeabilized cells)

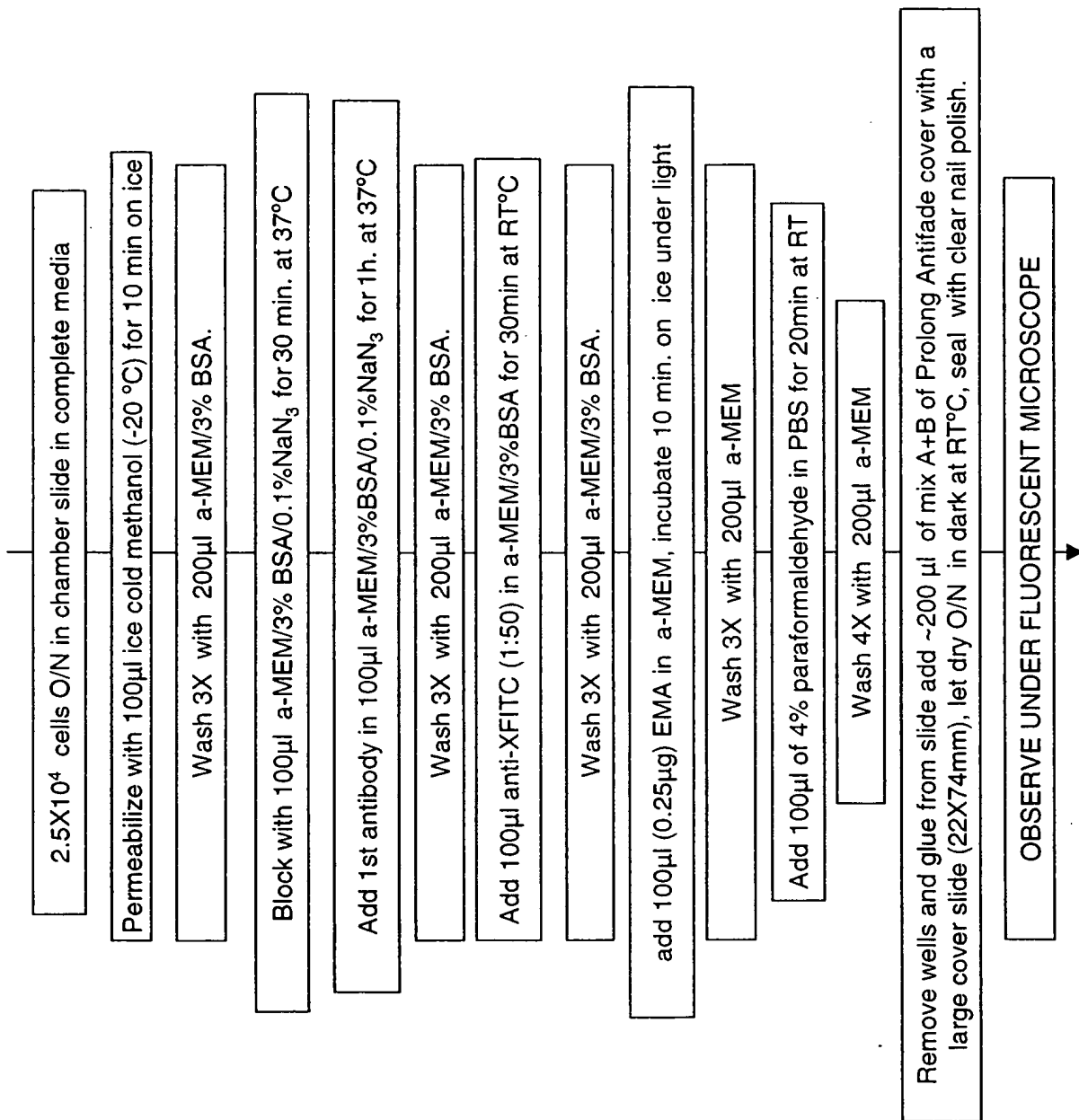


Figure 16: Immunofluorescence of surface exposed HSC70

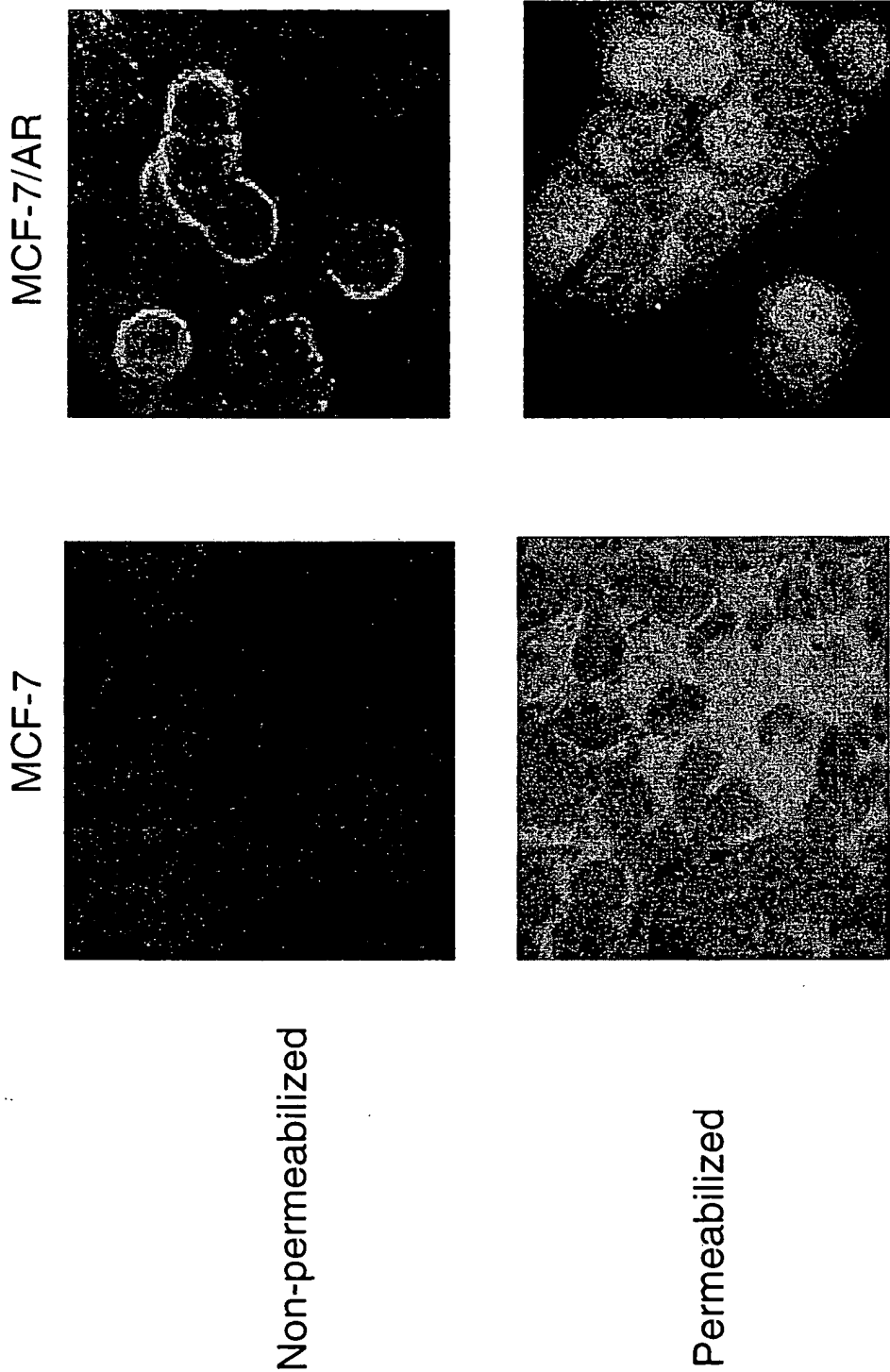
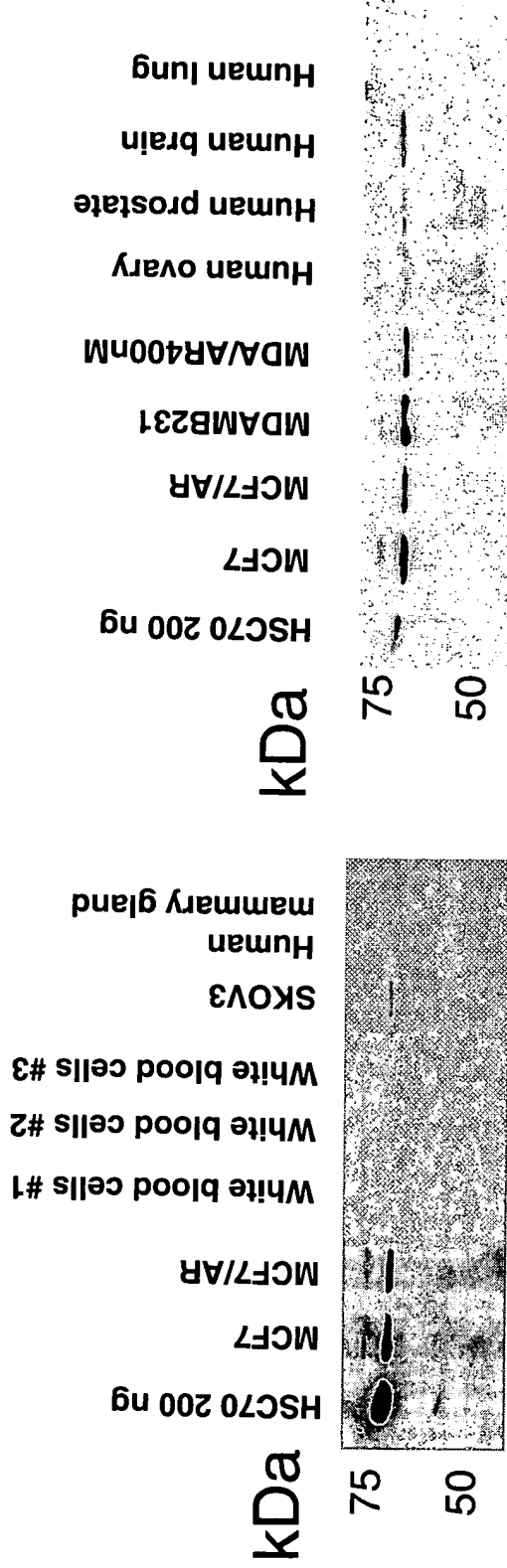


Figure 17: HSC70 expression



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Figure 18

